VIRAL CLEARANCE BY LOW PH HOLD

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/023,154 filed May 11, 2020 which is herein incorporated by reference.

[0002] The present invention generally pertains to methods for inactivating viral particles in protein sample using a low pH hold step. A statistical design of experiment incorporating several factors is used to evaluate and characterize the impacts of a low pH hold step for virus inactivation.

BACKGROUND

[0003] Biological products are susceptible to contamination (both endogenous contaminants and adventitious (coming from external sources) contaminants) from bacteria, fungi and viruses. Viral clearance, for example, viral inactivation, is an important step during the manufacture of biopharmaceutical products produced using mammalian cell lines. Global health authorities require evaluation of viral clearance for manufacturing biologics or biotechnology products, since viral contamination can be amplified during the growth of mammalian cell culture. Effective viral clearance studies are an important part of process validation, which also are important to ensure drug safety. Viral contamination also can affect raw materials, cell culture processes, bioreactor and downstream purification processes.

[0004] Viral validation studies are designed to document selected operating conditions regarding product quality to assure viral safety. The experimental design of viral clearance studies includes characterizations of the manufacturing process to identify significant development factors to improve understanding of processing conditions and justify selection of worst-case conditions. The processes of virus inactivation or removal include pH treatment, heat treatment, solvent/detergent treatment, filtration or chromatography. The mechanism of virus inactivation for low pH incubation includes a pH-based chemical reaction causing irreversible denaturation of surface glycoproteins or disruption of the lipid envelope of the virus.

[0005] The pH conditions adapted in manufacturing processes of the biopharmaceutical products may not be effective for virus inactivation. However, the pH required for viral inactivation can be significantly different than the pH ranges used in other manufacturing conditions. Using low pH incubation to obtain effective virus inactivation in protein samples is challenging for manufacturing biopharmaceutical products, since the low pH exposure of biopharmaceutical products can alter the quality or stability of proteins. It will be appreciated that a need exists for methods to evaluate and characterize the impact of a low pH hold step for virus inactivation during the manufacturing of biopharmaceutical products. These methods should provide effective and robust experimental designs to ensure virus inactivation for designing a manufacturing process, such as a purification process.

SUMMARY

[0006] The present application provides methods for viral clearance using low pH hold based on a statistical design of experiments incorporating several factors to evaluate and characterize the impacts of a low pH hold step for virus inactivation. The statistically designed experiment is used to evaluate the effect of pH conditions, ionic strength conditions, protein isotype, temperature, acid titrant, spike timing, and post-spike filtration on virus inactivation. These methods can be used to predict effective clearance when the viral inactivation step is conducted in the range of about pH 3.60-3.90 by, for example, manipulating ionic strength of the low pH starting material.

[0007] This disclosure provides a method for purifying a peptide or protein, such as an antibody, from a sample comprising one or more impurities including viral particles. In some exemplary embodiments, the method of the present application comprises: adjusting an ionic strength condition of the sample, adjusting a pH condition of the sample to an acidic pH, and subsequently maintaining the sample at the ionic strength condition and the pH condition for at least about 15 minutes to inactivate a quantity of viral particles; wherein the sample comprises one or more impurities including the viral particles. In one aspect, the quantity of the viral particle inactivation is at least about 3 LRF (logarithmic reduction factor) for using the method of the present application. In one aspect, the quantity of the viral particle inactivation is at least about 4 LRF for using the method of the present application.

[0008] In one aspect, the pH condition of the sample in the method of the present application is less than or equal to about pH 3.90. In one aspect, the pH condition of the sample is in a range of from about pH 3.60 to about pH 3.90. In another aspect, the pH condition of the sample is in a range of from about pH 3.65 to about pH 3.80. In another aspect, the peptide or protein in the sample is an antibody produced in a host-cell. In yet another aspect, the sample in the method of the present application is maintained at the ionic strength condition and the pH condition of the sample for at least about 30 minutes to inactivate the quantity of the viral particles. In one aspect, the sample in the method of the present application is maintained at the ionic strength condition and the pH condition of the sample for from about 15 minutes to about 30 minutes to inactivate the quantity of the viral particles.

[0009] In one aspect, the method of the present application further comprises optimizing the inactivation of the quantity of the viral particles by running a D-Optimal design of experiment. In another aspect, the D-Optimal design of experiment evaluates the following factors: the pH condition of the sample, and the salt concentration added to the sample. In one aspect, the D-Optimal design of experiment further evaluates the following factors: a type of the peptide or protein, a temperature of the sample, an acid titrant to adjust the pH condition of the sample, a spike timing for spiking the viral particles to the sample, or a presence of a post-spike filtration.

[0010] In one aspect, the sample in the method of the present application is an eluent from protein A chromatography. In another aspect, the ionic strength of the sample is adjusted using an addition of sodium chloride, wherein a concentration of the sodium chloride is in a range of from about 1 mM to about 100 mM, from about 1 mM to about 500 mM, about 25 mM, about 50 mM, about 72 mM, about 82 mM, about 100 mM, about 125 mM, about 150 mM, about 175 mM, or about 200 mM. In one aspect, the pH condition of the sample is adjusted using phosphoric acid or glycine HCl. In another aspect, the peptide or protein in the sample of the method is an antibody having an IgG1 isotype